Attachment 1

Standard Operating Procedure

I. PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to describe the procedure for purifying, quantifying and analyzing the virus-like particles (VLPs) of a filovirus strain.

II. RESPONSIBILITY

It is the responsibility of the person performing the assay to ensure that it is carried out according to the SOP and that all relevant documents are complete and accurate.

III. MATERIALS

VLP supernatants
Sterile 450-500mL VLP bottles
Sterile PBS
Sterile Microtubes
Laboratory Grade Sucrose to be dissolved into TNE buffer
TNE buffer (http://www.thelabrat.com/protocols/14.shtml)
13.2mL tubes rated to 50k RCF
50mL tubes rated to 30k RCF

IV. EQUIPMENT

Superspeed and Ultracentrifuges
JLA-10.5 Rotor (or equivalent that can hold 450mL bottles)
SW-41 and SW-32 Ultracentrifuge rotors (or equivalent)
Gradient Maker
Biological Safety Cabinet

V. PROCEDURE

A. VLP Purification Protocol:

1. Pour VLP Supernatants into sterile VLP bottles, balance and spin to pellet VLP's for 2-3 hrs at 7,000 RPM (~9,000 G). 400-450 mL per bottle in JLA-10.5 lite rotor.

2. Dissolve sucrose in TNE buffer, making a 10% and a 60% solution.

3. Decant supernatant under hood and re-suspend each pellet in 0.4-1.0 mL sterile PBS.

4. Make a 10-60% sucrose gradient using sterile sucrose in 13.2 mL Ultra-Clear tubes with Gradient Maker.

5. Carefully place re-suspended pellet on top of gradient, balance and ultracentrifuge for 16-20 hrs at 36,000 RPM at 4 degrees C (using sw-41 rotor).

6. Remove cloudy white band (should be approximately in the center). Place layer into 50 mL ultracentrifuge tubes, add sterile PBS to the top. Balance and Ultracentrifuge for 2 hrs at 26,000 RPM (SW-32 rotor) to pellet purified VLP's.

7. Discard supernatant and re-suspend pellet in Sterile PBS.

8. Irradiate at 50,000-100,000 rads.

9. Perform Bradford Analysis, add appropriate amount of sterile PBS so VLP is at a concentration of 2 mg/mL.

10. Analyze via Western Analysis of VLP samples for proteins of interest.

B. Bradford Protocol:

1. 5 uL VLP sample + 5 uL lysis buffer.

2. Incubate on ice (-20 degrees C) for 20 minutes.

3. 5 uL of Standard + 5 uL of lysis buffer for each standard.

4. Blank consisting of 10 uL of lysis buffer.

5. Add 990 uL of 1x Bradford Dye to each sample, standard and blank tube.

6. Read each sample on Spectrophotometer, creating new standard curve.

C. Western Analysis:

Monoclonal Antibodies Used for VLP- Western Analysis: All at 1:2500 concentration.

Ebola GP- 6D8-1-2
Ebola NP – Z-BC04-BC10-2 or 4AE8A1
Ebola VP40 – B-MD04-BD07-AE11

Marburg GP – 5D7-1-1
Marburg NP – 5F8-1-1
Marburg VP40- 1H11-1-1

HRP or AP anti-Mouse Secondary used at 1:1000 concentration.